

Supplement to Figure 2; (2-S1). Measurements for conformations and positions in Structures I through V.

Structure PDB ID Reference	80S•IRES 3J6Y (3J6X) Koh <i>et al.</i> (2014)	80S•IRES•eEF2•GDP•sordarin This work					80S•1tRNA 3J77	80S•2tRNAs 3J78
		I	II	III	IV	V	Svidritskiy <i>et al.</i> (2014)	
Position of PKI on the 40S	A site	Between A and P sites					P site	Reference Structures (no IRES)
IRES translocation state	INITIATION (no eEF2)	PRE- translocation (+ eEF2)	MID- translocation (+ eEF2)	MID- translocation (+ eEF2)	MID- translocation (+ eEF2)	POST- translocation (+ eEF2)	tRNA PRE- translocation (no eEF2, with one P/E tRNA)	tRNA POST- translocation (no eEF2, with two tRNAs)
Intersubunit rotation 40S rotation relative to 60S	Mid-rotated 3.7° (7.2°)	Fully rotated 9.6°	Mid-rotated 4.6°	Mid-rotated 4.5°	Almost non- rotated 1.3°	Least rotated 0.5°	Fully rotated 40S 11.0°	Non-rotated 40S 0°
40S Head swivel Beak rotation toward P-site	Mid - swiveled 10.1° (9.7°)	Mid-swiveled 11.6°	Most swiveled 17.1°	Most swiveled 17.1°	Mid - swiveled 13.8°	Least swiveled 1.1°	Non-swiveled 40S-Head 2.3°	Non-swiveled 40S-Head 0°
IRES conformation Distance between PKI and SL4, at phosphates U6913 – A6848	Extended 47 Å (47 Å)	Partially bent 36 Å	Partially bent 34 Å	Highly bent 28 Å	Highly bent 26 Å	Extended 44 Å	-	-
Distances	PKI and eEF2 position relative to the P-site stacking platform (U1191 and C1637)							
PKI to Body P site G6905, C4 – C1637, N1	16 Å (18 Å)	13 Å	7 Å	6 Å	5 Å	4 Å	-	-
PKI to Head P site G6905, O4' – U1191, O4'	23 Å (21 Å)	19 Å	18 Å	11 Å	8 Å	4 Å	-	-
eEF2 tip to Body P site H657, Cα – C1637, O4'	-	35 Å	27 Å	27 Å	26 Å	25 Å	-	-
eEF2 tip to Head P site H657, Cα – U1191, O4'	-	41 Å	38 Å	37 Å	32 Å	24 Å	-	-
Distances	eEF2 position relative to A site (post-translocation eEF2 in Structure V)							
Domain IV H657, Cα – H657, Cα (V)	-	11 Å	4 Å	5 Å	2 Å	0 Å (Reference)	-	-
Domain I E265, Cα – E265, Cα (V)	-	18 Å	9 Å	8 Å	2 Å	0 Å (Reference)	-	-